Organic microcontaminants in tomato crops irrigated with reclaimed water grown under field conditions: occurrence, uptake and health risk assessment

A.B. Martínez-Piernas,[†] P. Plaza-Bolaños,[†] P. Fernández-Ibáñez, [‡] A. Agüera*, [†]

[†]*CIESOL (Solar Energy Research Center), Joint Centre University of Almeria-CIEMAT, Carretera de Sacramento s/n, E-04120, Almeria, Spain*

[‡]*Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster, Newtownabbey, BT37 0QB, Northern Ireland, United Kingdom*

*Corresponding author

Email: aaguera@ual.es, Tel: +34 950 015 531, Fax: +34 950 015 008

ORCID: 0000-0003-2649-6772

ABSTRACT

In many regions reuse of reclaimed water (RW) is a necessity for irrigation. Presence of organic microcontaminants (OMCs) in RW and their translocation to plants may represent a risk of human exposure. Nevertheless, information available about real field crops is scarce and focused on a limited number of compounds. The novelty of this work relies on the application of a wider-scope analytical approach based on a multi-analyte target analysis (60 compounds) and a suspect screening (1300 compounds). This methodology was applied to real field-grown tomato crops irrigated with RW. The study revealed the presence of 17 OMCs in leaves (0.04 - 32 ng g^{-1}), and 8 in fruits (0.01 - 1.1 ng g^{-1}); 5 of them not reported before in real field samples. A health-risk assessment, based on the toxicological threshold concern (TTC) concept, showed that RW irrigation applied under the conditions given do not pose any threat to humans.

KEY WORDS

Organic microcontaminants

Plant uptake

Reclaimed water reuse

Health risk assessment

LC-MS target/suspect analysis

INTRODUCTION

The lack of fresh water resources for agriculture in arid and semiarid regions is a worldwide problem that needs to be addressed in the 21st century. Factors such as climate change and increasing population have led to severe droughts in areas where intensive agriculture is the main economic activity. The reuse of reclaimed water (RW) for agriculture irrigation seems to be an excellent approach to deal with water scarcity,[1–5] since it not only promotes efficient water usage, but also has other advantages such as reducing the application of fertilizers and avoiding the discharge of waste into natural water bodies, thus contributing towards the preservation of the environment.[6]

In Europe, the Mediterranean area is heavily influenced by low and irregular rainfall, a fact that has worsened water shortages leading to a lower water supply for agricultural purposes mainly during peak water demand periods. Nowadays, countries such as Cyprus, France, Greece, Italy, Portugal and Spain, have adopted regulations regarding the reuse of RW for crop irrigation due to the increasing application of this practice.[7] So much so, in Spain, the 10.8% of the RW is reused, being the 71% of it destined to agriculture.[7] In most cases, the national regulations include specific threshold values for either microbiological (e.g. *E. coli*, intestinal nematodes) and physical-chemical parameters (e.g. total suspended solids, turbidity) for any restricted use,[8] being more strict for agricultural uses. The European Commission has recently launched a proposal for a regulation on minimum requirements for water reuse, which includes recommendations based on a health and environmental risk management framework for future water reuse legislation.[9] Again, only microbiological and physical-chemical parameters have been considered. However, in the last decade, the presence of organic microcontaminants (OMCs) in RW, which are not completely removed during the treatments,[10] have been pointed out as a potential risk. It has been demonstrated that intensive use of RW in

agriculture leads to their accumulation in agricultural soils[11,12] and their subsequent uptake by plant roots, in some cases being able to translocate to aerial parts of plants such as leaves and fruits through the vascular plant system.[2,13–15] However, some knowledge gaps and the lack of reliable data still prevent to make definite conclusions about their risk posed to humans and the environment.

Numerous studies have shown translocation of OMCs to edible parts of crops in simulated or controlled conditions.[13–21] Nevertheless, little is known about their occurrence and accumulation in real field crops exposed to RW irrigation for long time periods. Recently, Picó et al.[14] have evaluated the accumulation of OMCs in agricultural soils and crops irrigated with treated wastewater, finding up to 6 pharmaceuticals in different crops as cabbage, green beans or eggplants. Also Riemenscheneider et al.[5] reported the translocation in real field samples of 12 micropollutants and metabolites to different plant organs such as roots, stems, leaves and fruits of 10 different vegetables irrigated with river water mixed with effluent from a wastewater treatment plant (WWTP). In another study, Wu et al.[2] monitored the accumulation of 19 OMCs in 8 vegetables irrigated with RW showing a detection frequency of 64% at concentrations in the range of 0.01-3.87 ng g^{-1}, dry weight, (d.w.).

However, most of the reported studies analyze a low number of compounds or are focused on certain pharmaceutical classes. In order to obtain a comprehensive evaluation of the impact of OMCs in the food chain, it is necessary to apply multi-analyte/class methodologies able to provide qualitative information for a wide range of compounds, given the large number of OMCs reported in RW. Therefore, in addition to wider target methods, non-target screening methodologies based on high resolution mass spectrometry (HRMS) should be applied, leading to the identification of substances outside the limited scope of the target analysis.[12,14] This approach should contribute towards improving data

available regarding the occurrence/accumulation of OMCs in final products intended for human consumption to ensure safe use of RWW in terms of health risk assessment.

Finally, the reported accumulation of OMCs in crops is in general low and no risk for public health is expected to be associated to the until now, few known individual compounds in crops grown under real field conditions.[22,23] However, further work needs to be carried out to assess the risk of not previously evaluated compounds that are present in the edible tissues of plants grown under long-term and continuous exposition to these microcontaminants.[24] This data will be valuable to study the risk associated with mixtures of OMCs in end-products in future works.

The goal of this work was to increase the current information about the translocation of OMCs derived from reuse by providing reliable data on their occurrence and fate in real tomato crops (leaves and fruits) after long-term exposure to RW irrigation practices under field conditions. Field-grown tomato plants were cultivated in agricultural soils previously analyzed[12] and irrigated with RW for more than 10 years without soil substitution. With this aim in mind, a combined strategy based on a multi-analyte target analysis (including 60 compounds considered as contaminants of emerging concern) together with a suspect screening methodology (covering a list of 1300 potential contaminants) was applied. A simple and quick QuEChERS-based method was used for sample preparation and liquid chromatography coupled to low and high resolution mass spectrometry, were selected. A health-risk assessment approach was also applied to evaluate human exposure of the RW-derived OMCs in tomato fruits.

MATERIALS AND METHODS

Chemicals and Reagents. A total of 60 OMCs (mainly pharmaceuticals from a variety of therapeutic classes) (Table S1) were analyzed due to their frequent identification in

WWTP effluents.[10] All reference standards (purity > 98%) were acquired from Sigma-Aldrich (Steinheim, Germany). Methanol (MeOH), acetonitrile (ACN), water, formic acid and acetic acid (LC-MS grade) were obtained from Sigma-Aldrich. Ultrapure water for LC-MS/MS analysis was produced using a Milli-Q water purification system from Millipore (Darmstadt, Germany). For the QuEChERS extraction method, anhydrous magnesium sulfate ($MgSO_4$) and sodium acetate (NaOAc) were purchased from Sigma Aldrich (all purity > 98%). Octadecyl-silyl-modified silica gel (C18) and primary-secondary amine (PSA) were acquired from Supelco (Bellefonte, PA, USA).

Stock standard solutions of each compound were prepared at 1000-2000 mg L^{-1} in MeOH. Multi-compound working solutions were prepared at a concentration of 10 mg L^{-1} in MeOH by diluting the individual stock solutions. All standard solutions were stored in amber glass vials at -20°C. Matrix matched calibration solutions were daily prepared and used for quantification purposes. Two surrogate standards, carbamazepine-d_{10} and ^{13}C-caffeine, were used to check the extraction efficiency.

Sample Collection. To study the occurrence and distribution of OMCs in the plant system, three greenhouses were selected (GH1, GH2 and GH3; intensive production; 13000–25000 m^2), in which two different tomato varieties, ramyle (GH1, GH2) and retinto (GH3) were grown. A fourth greenhouse dedicated to the experimental soilless culture (SP1) of the cherry tomato variety, which was grown in pots filled with perlite substrate, was also included in the study. All greenhouses were located in Almeria province (Spain) and had been irrigated with RW for no less than ten years without soil replacement. The RW was provided by a regeneration plant facility which treats municipal wastewater secondary effluents by filtration (sand and anthracite filters) and chlorination (NaClO). Treated water fulfilled the requirements of water quality according to the Spanish regulation for water reuse.[8] Drip irrigation was employed in all

greenhouses. Four sampling events during the commercial tomato campaign took place from January (full plant growth) to May 2016 (removal of tomato plants). In each sampling event, tomatoes at a mature stage of growth and leaves of tomato plant samples (500 g in each case) of similar size were taken from different parts of the greenhouse following a W sampling route. The subsamples were chopped and mixed to form a homogeneous composite sample and were kept in the dark at -20°C until their analysis. Three replicates of each sample were extracted for quantification purposes. RW was analyzed coinciding with the first sampling of tomato fruits and leaves.

Sample Extraction. The extraction of OMCs in tomato fruit and leaves was carried out by a modification of the QuEChERS acetate extraction method previously published by our group.[13] Briefly, a portion of 10 g of plant material were placed into a 50-mL polypropylene centrifuge tube. After that, 10 mL of 1% acetic acid in ACN and 20 µL of the extraction quality control solution were added to the sample and the tube was shaken for 5 min and centrifuged at 3500 rpm (2054x*g*) for 5 min. Following the extraction procedure, a clean-up step was carried out. An aliquot of 5 mL of the upper organic layer was transferred to a 15-mL centrifuge tube containing 750 mg of anhydrous $MgSO_4$, 125 mg of primary-secondary amine (PSA) and 125 mg of C18. Then the tube was shaken for 30 s in a vortex and centrifuged at 3500 rpm for 5 min. Following this, the extract (4 mL) was transferred to screw-cap vials adding 10 µL of ACN at 1% of formic acid per milliliter of extract. Prior to injection into the LC-MS/MS system, 100 µL of the extract was evaporated and reconstituted in 100 µL of ACN:H_2O (10:90, *v/v*).

Liquid Chromatography-Mass Spectrometry. *LC-MS/MS Target Analysis.* The HPLC system (Agilent Series 1200, Agilent Technologies, Palo Alto, CA, USA) consisted of a binary pump, a degasser and an autosampler. Chromatographic separation was accomplished using a XDB C18 (50 x 4.6 mm, 1.8 µm particle size) column (Agilent

Technologies). Mobile phases were 0.1% formic acid in MilliQ water (solvent A) and ACN (solvent B). The gradient used ranged from 10% to 100% of solvent B: initially it was kept at 10% for 1 min, increased from 10% to 50% over 3 min and from 50% to 100% over 10 min; kept at 100% for 4 min and finally returned to its initial conditions. The total analysis run time was 18 min. The injection volume was 10 μL and the flow rate was set to 0.4 mL min^{-1}. The column outlet system was connected to a hybrid triple quadrupole-linear ion trap-mass spectrometer 5500 QTRAP® (Sciex Instruments, Foster City, CA, USA) equipped with an ESI source (TurboIon Spray) operating with positive and negative polarities. The ionization settings used were: ionspray voltage, 5000 V; curtain gas, 25 (arbitrary units); GS1, 50 psi, GS2, 40 psi; and a temperature, 500 °C. Nitrogen was used as a nebulizer, curtain and collision gas. The multiple reaction monitoring (MRM) mode was chosen for the analysis of the target compounds. To increase the sensitivity for the acquisition performance, the schedule MRM™ algorithm was applied with a retention time window of 40 s per transition. The optimal mass spectrometric parameters for each compound are summarized in Table S2. Sciex Analyst version 1.6.2 software was applied for data acquisition and processing, and MultiQuant 3.0.1 software for data quantification.

LC-QTOF-MS/MS Suspect screening analysis. Chromatographic separation was performed using a HPLC (Agilent 1260 Infinity system) equipped with a Poroshell 120 EC-C18 (50 x 4.6 mm, 2.7 μm particle size) analytical column (Agilent Technologies). 0.1% formic acid in ultrapure water (solvent A) and ACN (solvent B) were used as mobile phases. The injection volume was 20 μL and the flow rate was 0.5 mL min^{-1}. The gradient used ranged from 10% to 100% of solvent B: initially it was kept constant at 10% for 2 min, then increased linearly from 10% to 100% for 9 min and finally it remained constant for 4 min before being returning to initial conditions. The total analysis run time was 22

187 min. The LC system was coupled to a QTOF mass analyzer Triple TOF 5600+ (Sciex
188 Instruments), with a DuoSpray^{TM} ion source consisting of an electrospray (ESI) interface
189 for sample injection and an atmospheric-pressure chemical ionization interface (APCI)
190 for calibrant delivery. Samples were analyzed in ESI+ and ESI− modes. The ESI source
191 parameters were: ionspray voltage, 4500 V; curtain gas, 25 (arbitrary units); GS1, 60 psi;
192 GS2, 60 psi; and temperature, 575°C. Nitrogen served as a nebulizer, curtain and collision
193 gas. The equipment worked via TOF MS survey scan (resolving power of 30000) with an
194 accumulation time of 250 ms followed by four IDA (Information Dependent Acquisition)
195 TOF MS/MS scans with an accumulation time of 100 ms. The IDA feature allows the
196 performance of MS/MS acquisitions simultaneously with the MS acquisition. The *m/z*
197 range was from 100 to 2000. IDA criteria considered dynamic background subtraction.
198 Collision energy of 30 eV with a ± 15 eV spread was applied for MS/MS fragmentation.
199 Diverse Sciex software (Analyst TF 1.5, PeakView™ 2.2 and MasterView 1.1) was used
200 to record and process LC-QTOF-MS/MS data. A suspect list containing 1300 OMCs
201 commonly found in WWTP effluents was made before sample processing. The settings
202 considered for a final confirmation of the compounds were: a) a mass accuracy error for
203 the precursor ion < 5 ppm; b) an isotope ratio difference < 10%; c) a MS/MS spectra fit
204 ≥ 80% when the acquired spectra was compared with the MS/MS spectra of the standard;
205 and d) a difference of ± 0.1 min in the retention time (RT) when it was compared with
206 the standard in matrix.

207 **Method Validation.** Concerning the quantitative method for tomato fruits and leaves,
208 the present methodology was validated assessing trueness (in terms of recoveries),
209 precision (expressed as relative standard deviation, RSD), linearity and limits of
210 quantification (LOQs). For method validation, tomato leaves and fruits not irrigated with
211 RW were used as blank matrices. Triplicate analyses of samples spiked at 0.5 ng g^{-1} were

212 used to calculate the recoveries. Satisfactory mean recovery values were considered in
213 the range 70-120% with an associated precision RSDs ≤ 20%. The linearity was studied
214 by matrix-matched standard calibration curves at six concentration levels ranging from
215 0.01 to 10 ng g^{-1}. Linearity was considered as acceptable when the determination
216 coefficients (R^2) were ≥ 0.990. The LOQs were set as the lowest acceptable concentration
217 in the matrix-matched calibration curve which yielded the signal-to-noise (S/N) ratio
218 closer to 10 for the quantification transition (SRM 1). The quantification of the analytes
219 present in the samples was carried out by matrix-matched calibration curves of all
220 validated compounds. OMCs quantified in real samples fulfilled the requirements for
221 recoveries, precision and linearity (Table S3).
222 Regarding RW, the sample collected was analyzed per triplicate by direct injection
223 following the methodology reported elsewhere,[10] which was previously validated for the
224 analysis of 115 OMCs in WWTP effluents.
225 **Health-risk Assessment.** The health risk associated with presence of OMCs in tomato
226 fruits was estimated using the threshold of toxicological concern (TTC) approach. This is
227 useful for assessing the risk involved with substances present in food at low
228 concentrations and for which toxicity data is still scarce.[25] TTC has previously been
229 applied to the risk assessment of OMCs in crops.[3,20] In this study, an average body weight
230 of 70 kg for adults and 12 kg for toddlers was considered for the estimation of daily
231 consumption. The TTC values and compounds classification were estimated based on the
232 well-known Cramer classification tree. The Cramer method mainly utilizes chemical
233 structures and evaluates the total human intake to establish priorities for testing.[26] This
234 protocol considers a number of factors related to the presence of the chemical component
235 under study or the frequency of ingestion, including: a) different metabolic pathways for
236 either activation or deactivation of the chemicals under study; b) partial presence of a

target substance in a variety of standard foods and their metabolites; c) toxicity data for each substance; and d) the level of exposure to humans via oral ingestion. This information is then managed to obtain the TTC value of each compound in terms of µg-ng kg^{-1} body weight (b.w.) day^{-1}.[27] For the OMCs translocation that were not reported before, we considered as minimum tolerated exposure of each OMC as equals to the TCC value given for the parent compound (Houeto et al, 2012; Munro et al., 1996; Stanard et al., 2015).

TCC values and compound classification were estimated using ToxTree software (ToxTree v.3.1.0, by JRC Computational Toxicology and Modeling and developed by Ideaconsult Ltd, Sofia, Bulgaria). The TTC values for all the compounds under study were determined for the highest CEC concentrations of all greenhouses (SP1, GH1, GH2, GH3) obtained in each sampling event (S1 - S4). Statistical analysis of all the samples and repeated measurements in pairs ($p < 0.05$) were performed using ANOVA analysis.

RESULTS AND DISCUSSION

Method validation results. The proposed methodology was validated in tomato fruits and leaves of tomato plant for a total of 60 OMCs. The validation results are presented in Table S3.A total of 48 out of 60 compounds (80%) in fruit and 31 (51%) in leaves showed acceptable recoveries in the 70-120% range with RSD ≤ 20%. Tomato results are in line with the previous method validation of the same compounds in other vegetable matrices such as lettuce, radish and strawberry.[13] However, the number of successfully recovered OMCs in leaves was lower than in fruits probably due to the complexity of this matrix. The high content of chlorophylls and pigments may suppress OMCs extraction efficiency in leaves case. In general, very low RSD values under 10% were found in the majority of the cases regardless the recovery value. Solely for loratadine in leaves it was obtained a

RSD out of the acceptable values. This demonstrates the repeatability of the method. All compounds presented good linearity with R^2 values higher than 0.991 and LOQs ranged from 0.01 to 2 ng g^{-1}; showing more than the 50% of the analytes low LOQs below 0.1 ng g^{-1} in both commodities. In spite of some OMCs such as clotrimazole, fenoprofen or sulfapyridine do not fulfill the acceptable criteria for validation; they were maintained in the method for qualitative purposes. Only those OMCs for which the method could be fully validated adopting the criteria aforementioned were quantified in real samples.

OMCs in Irrigation Water. An analysis of the irrigation water was carried out at the beginning of the study to obtain an overview of the potential exposure of the crops to the tested OMCs. As can be observed in Table S4, up to 51 OMCs could be identified at concentration values ranging from 15 to 14424 ng L^{-1}. The metabolites of dipyrone, 4-FAA and 4-AAA (14424 and 5396 ng L^{-1}, respectively), the diuretics hydrochlorothiazide and furosemide (2758 and 1694 ng L^{-1}, respectively), and the beta-blocker atenolol (1279 ng L^{-1}), were detected at the highest concentrations. It was expected that OMC concentrations in RW would vary throughout the study. However, overall these results are in line with previous monitoring studies carried out on urban WWTP effluents from Almeria[10,13] and can be considered as representative of the type/concentration of compounds usually present in the RW. The presence of 35 of these compounds has also been previously reported by our group in soil and soilless perlite substrate samples taken from the greenhouses monitored, which show average concentrations in the range 0.14 - 99 ng g^{-1}, d.w. (Table S4). Although the presence of OMCs in irrigation water and soils cannot be directly related to their occurrence in plant tissues due to the diverse factors involved in plant uptake, it can be assumed that their availability to be taken up by roots and translocate to edible parts is feasible when RW is used in irrigation.

Occurrence of OMCs in Tomato Plant Leaves. Greater knowledge about the occurrence of OMCs in vegetables irrigated with RW under field conditions is key to evaluating the quality of crops and determining potential consequences of reusing RW in agriculture irrigation. Moreover, the analysis of non-edible parts of the tomato crop, such as leaves, which may be used as sustenance for livestock feeding, is also important since it could represent another pathway for human exposure to OMCs. In this study, a total of 60 target compounds (Table S1) were monitored in real samples of tomato and tomato plant leaves to evaluate their distribution throughout the plant-fruit system.

The average concentration levels of OMCs found in leaf samples during the four sampling events are shown in Table 1. Up to 17 CECs were detected in leaves with average concentrations ranging from 0.04 to 32 ng g^{-1} wet weight (w.w.). The compounds that eventually reached the higher concentrations were the metabolites of dypirone, 4-AAA and 4-FAA (11 and 32 ng $g^{-1,}$ respectively), the anticonvulsant drug carbamazepine (8.9 ng g^{-1}), its metabolite carbamazepine epoxide (8.1 ng g^{-1}) and the antidepressant venlafaxine (4.0 ng g^{-1}). Regarding the frequency of detection, only 7 OMCs were found in all samples, namely caffeine, paraxanthine, carbamazepine, carbamazepine epoxide, hydrochlorothiazide, mepivacaine and venlafaxine; evidencing their higher capability of uptake and translocation within the plant. Nevertheless, their concentrations did not increase during the sampling period; a fact that could demonstrate stable accumulation despite constant irrigation with RW. Another group of OMCs were detected at very low concentrations (<LOQ) and/or showed low frequency of detection. This was the case for acetaminophen, antipyrin, diazepam, propranolol and the antibiotic trimethoprim.

In addition to the target analysis, samples were retrospectively analyzed by the acquired LC-QTOF-MS/MS sample information. The strategy allowed the identification of 3 other OMCs: flecainide, lidocaine and tramadol (Figures S1-S3). These compounds were also

311 found in the irrigation water and soil samples (Table S4).[12] Almost all of them were
312 identified in every sampling event, showing uptake from soil to leaf plant tissues. As the
313 methodology could not be validated for these analytes, estimated concentration values
314 had to be calculated (Table 1).
315 In general, no significant differences considering concentration levels were found
316 between the different tomatoes produced in the greenhouses. This suggests there is no
317 correlation between plant uptake and the tomato plant variety.
318

Table 1. Average OMC concentrations (ng g^{-1}, w.w.) quantified in tomato plant leaves

	SP1[a]				GH1[b]				GH2				GH3			
Compound	**S1[c]**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
4-AAA	<LOQ[d]	-	0.5	<LOQ	<LOQ	-	-	<LOQ	0.4	-	<LOQ	<LOQ	<LOQ	<LOQ	12	<LOQ
4-FAA	8	n.d.[e]	32	13	n.d.	n.d.	7	5	<LOQ	n.d.	4	3	<LOQ	3	4	2
Acetaminophen	n.d.	n.d.	<LOQ	2	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	3	n.d.	n.d.	n.d.	3
Antipyrine	<LOQ	<LOQ	1	<LOQ	<LOQ	<LOQ	0.7	n.d.	<LOQ	<LOQ	0.6	n.d.	<LOQ	<LOQ	<LOQ	n.d.
Caffeine	0.5	1	0.7	0.5	0.5	1	0.4	<LOQ	0.4	1	0.5	<LOQ	0.5	1	0.5	<LOQ
Carbamazepine	5	5	5	2	3	6	5	2	2	9	3	6	6	4	7	4
Carbamazepine epox	3	3	2	3	3	2	0.7	4	2	2	0.5	8	4	2	1	8
Flecainide[f]	n.d.	2	n.d.	0.9	n.d.	2	4	4	2	4	4	4	3	2	3	4
Diazepam	<LOQ	<LOQ	0.06	0.04	<LOQ	<LOQ	<LOQ	0.01	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
Hydrochlorothiazide	1	1	1	0.6	1	1	2	1	0.9	1	0.9	0.6	1	0.6	1	0.6
Lidocaine[f]	1	2	10	8	3	8	6	11	3	5	6	6	7	6	4	8
Mepivacaine	0.6	0.5	0.5	0.3	0.8	0.7	0.8	0.9	0.6	1	0.6	1	1	0.6	0.3	0.8
Paraxanthine	0.2	0.6	0.4	0.3	0.2	0.6	<LOQ	<LOQ	0.2	0.5	0.3	0.2	0.2	0.3	<LOQ	<LOQ
Propranolol	<LOQ	<LOQ	<LOQ	<LOQ	0.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.4	<LOQ	<LOQ	<LOQ
Tramadol[f]	1	2	1	4	0.8	2	2	3	0.6	3	2	3	0.2	3	3	3
Trimethoprim	n.d.	n.d.	2	n.d.	n.d.	n.d.	0.7	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	n.d.	n.d.
Venlafaxine	2	1	2	0.7	2	2	2	3	2	4	3	4	4	2	2	4

[a]SP: soiless perlite culture; [b]GH: greenhouse; [c]S: sampling event; [d]<LOQ: concentration below the limit of quantification; [e]n.d.: not detected; [f]Estimated OMC concentrations quantified by LC-QTOF-MS/MS

323 Results obtained in the field study concerning translocation of selected OMCs via plant
324 roots to other plant tissues, confirm previous results reported in studies under controlled
325 conditions. For instance, Martínez-Piernas et al.[13] reported the accumulation of diverse
326 analytes such as 4-AAA, 4-FAA, caffeine, carbamazepine, carbamazepine epoxide,
327 hydrochlorothiazide, lincomycin, mepivacaine and venlafaxine, among others, in lettuce
328 and leaves of radish when RW was used as irrigation water. Wu et al.[16] compared the
329 concentrations found for a group of OMCs such as acetaminophen, caffeine,
330 carbamazepine and diazepam in roots and leaves of lettuce, spinach, cucumber and pepper
331 irrigated with spiked water. The metabolism and plant uptake of diazepam has also been
332 evaluated by Carter et al.[17] in radish and silverbeet cultivated with spiked soil. The
333 antibiotic trimethoprim has been reported by Dodgen et al.[18] as being translocated to
334 lettuce, carrot and tomato leaves in an experiment carried out under controlled conditions
335 of temperature and humidity. Other studies have investigated the impact of soil
336 composition in OMCs' plant uptake in leafy crops when they were cultivated with spiked
337 water, observing correlations between soil characteristics and root uptake.[19,20]
338 However, very few studies have analyzed real field samples exposed to OMCs. Wu et
339 al.[2] described the translocation of caffeine and carbamazepine within the different plant
340 organs in vegetables irrigated with RW and cultivated under field conditions. In addition,
341 Riemenschneider et al.[5] observed the accumulation of caffeine, carbamazepine,
342 carbamazepine epoxide and hydrochlorothiazide in different vegetables and agricultural
343 plant tissues. In another study, levels of lincomycin were reported up to 20 μg kg^{-1} (d.w.)
344 in leafy vegetables such as rape, celery and coriander grown in soil amended with
345 manure.[28]
346 **Occurrence of OMCs in Tomato Fruit.** Concentrations of OMCs were found to a
347 lesser extent in tomato fruits, these generally being 10 times lower in fruit compared to

leaves (Figure 1). A total of 12 OMCs were detected in tomato samples. However, only 8 compounds could be quantified in at least one sample (Table 2). In general, the compounds that showed higher frequencies of detection and concentrations in leaves were also present in tomatoes, showing mobility through the plant transpiration stream up to fruits. The highest concentration was observed for caffeine (1.1 ng g^{-1}), followed by the metabolite 4-AAA (0.4 ng g^{-1}), then carbamazepine (0.23 ng g^{-1}), hydrochlorothiazide (0.15 ng g^{-1}), venlafaxine (0.15 ng g^{-1}), mepivacaine (0.09 ng g^{-1}) and carbamazepine epoxide (0.07 ng g^{-1}). 4-FAA, acetaminophen, acetanilide and paraxanthine were identified at concentrations below the LOQ in at least one sample.

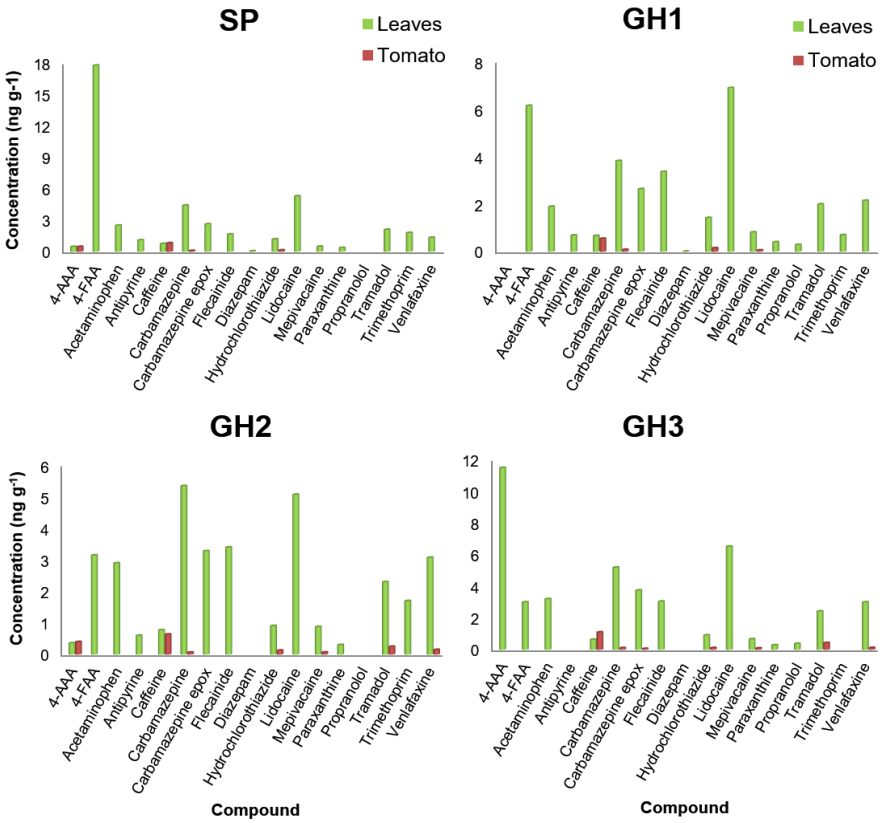

Figure 1. Average OMC concentrations found in leaves (green) and tomatoes (red) in each sampling site during the four sampling events.

The retrospective analysis of tomato fruit samples revealed the presence of a previously detected analyte in leaves by the same approach: tramadol (Figure S3). It is an opioid analgesic generally used for moderate and severe pain. Tramadol was found in tomatoes from two different greenhouses. Estimated concentrations of this compound are shown in Table 2.

No remarkable differences were found between the concentrations observed for cherry (SP1), ramyle (GH1, GH2) or retinto (GH3) tomato varieties. This fact evidences that despite the higher size of the last two types and the different agricultural practices (soilless culture for cherry and real soils for the rest), OMC accumulation was similar in all cases.

Table 2. Average OMC concentrations (ng g^{-1} , w.w.) quantified in tomato fruit samples

	SP1[a]				GH1[b]				GH2				GH3			
Compound	**S1[c]**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
4-AAA	-	<LOQ[d]	-	0.4	-	-	-	<LOQ	<LOQ	-	-	0.4	-	-	-	-
Caffeine	<LOQ	0.4	<LOQ	<LOQ	<LOQ	0.3	0.8	<LOQ	<LOQ	0.8	0.5	<LOQ	<LOQ	1	<LOQ	<LOQ
Carbamazepine	0.2	0.01	0.01	<LOQ	0.2	0.01	0.03	0.1	0.05	<LOQ	0.06	0.1	0.05	0.07	0.1	0.2
Carbamazepine epox	<LOQ	<LOQ	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.05	<LOQ	<LOQ	0.07
Hydrochlorothiazide	-	-	0.1	0.1	-	-	<LOQ	0.2	-	<LOQ	<LOQ	0.1	-	0.1	<LOQ	0.1
Mepivacaine	<LOQ	-	-	-	<LOQ	-	<LOQ	0.06	<LOQ	-	<LOQ	0.07	-	<LOQ	<LOQ	0.1
Tramadol [a]	-	-	-	-	-	-	-	-	-	0.2	-	-	-	0.1	-	0.7
Venlafaxine	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	<LOQ	<LOQ	<LOQ	0.1	<LOQ	<LOQ	<LOQ	<LOQ	0.1

[a]SP: soiless perlite culture; [b]GH: greenhouse; [c]S: sampling event; [d]<LOQ: concentration below the limit of quantification; [e]estimated OMC concentrations quantified by LC-QTOF-MS/MS

As was observed in leaves, caffeine and carbamazepine were detected in all greenhouses in every sampling event. Their plant uptake and translocation to the edible parts of vegetables is well described in literature.[2,13,29] Some studies have already reported them in tomato crops cultivated under field and controlled conditions.[5,21] Also metabolites such as carbamazepine epoxide have been identified in tomatoes when plants were irrigated with water spiked with carbamazepine under experimental conditions.[30] Hydrochlorothiazide has been reported in other vegetable tissues such as roots and leaves of parsley cultivated under field conditions[5] evidencing its high capability of translocation through the plant system. OMCs such as 4-AAA, 4-FAA, mepivacaine and venlafaxine, which were quantified in leaves, were also translocated to fruits and identified in certain sampling events. This group of OMCs has been found in the edible parts of lettuce and radish cultivated under controlled conditions submitted to RW irrigation.[13] Considering that 4-AAA and 4-FAA have exhibited toxicity,[31] it is important to monitor their occurrence and to evaluate their repercussions on human exposure.

To our knowledge, 4-AAA, mepivacaine, paraxanthine, tramadol and venlafaxine have not previously been identified either in plant tissues or edible parts of real field samples, which highlights the importance of applying wide-scope analytical methods for the evaluation of reuse of RWW in agriculture under different conditions and crops, and the potential of HRMS for the identification of non reported substances in environmental analysis. These results contribute to cover the gap of knowledge regarding the possible OMCs that can be present in edible parts of crops. This will help future studies dealing with the evaluation of the environmental and human risks associated with mixtures of analytes.

Accumulation in Plant Tissues and Properties of Compounds. It is well-known that OMCs' uptake by roots is accessible for those compounds that are dissolved in the

400 solution of the soil pore water. In general, neutral and cationic species in the soil solution
401 are susceptible to uptake by roots and subsequently translocate to the aboveground parts
402 of plants by the transpiration stream.[16,20,32] On the other hand, anions are considered less
403 transported to aerial parts due to their accumulation in cell roots by mechanisms such as
404 ion-trapping.[32] The translocation of OMCs from roots to other plant organs is possible
405 due to their capability of moving through the transpiration streams. This mobility depends
406 on diverse analyte physical-chemical properties such as lipophilicity (K_{ow}), pK_a or the
407 type of crop, among others.[3,33]
408 The results found in this study revealed that the OMC concentration values detected in
409 tomato leaves were significantly higher (up to ten times in some cases, Table 1) than those
410 found in tomatoes (Table 2). This behavior has been already reported in several
411 studies.[2,13,20,33] This issue is explained by the greater water flow to leaves, leading to
412 higher accumulation of OMCs in leafy parts than in fruits.
413 In Table S5, the diverse lipophilic coefficients (log K_{ow} for neutral compounds and log
414 D_{ow} for ions), pK_a and molecular charge (soil pore solution pH = 7.5) of the OMCs
415 identified in this work are shown. In general, moderate to strong bases ($pKa \geq 7$), in its
416 cationic species or partially ionized (flecainide, hydrochlorothiazide, lidocaine,
417 mepivacaine, propranolol, trimethoprim and venlafaxine); weak bases ($pK_a < 6$) in neutral
418 form (4-AAA, 4-FAA, antipyrine, caffeine, carbamazepine, carbamazepine epoxide,
419 diazepam and paraxanthine) and a very weak acid ($pK_a > 7.5$) in its neutral form
420 (acetaminophen) were detected. The fact that these analytes are neutral or cations for a
421 wide range of pH values explains their good distribution through the transpiration streams
422 (~5.5 < pH < ~7.5), being able to cross membranes, reaching leaves and fruits.[20] Although
423 some compounds were partially ionized, they were translocated via the transpiration-
424 derived mass flow subsequently being found in leaves, and in case of mepivacaine and

hydrochlorothiazide, in both leaves and fruits. No OMC in anionic form was detected in either leaves or tomato. This is in accordance with the aforementioned reasons about the expected low translocation of anions through the vascular system, making its distribution less possible through plant streams.

As shown in Table S5, log K_{ow} and log D_{ow} of the OMCs identified, ranged from low to medium lipophilic values ($-0.63 < \log K_{ow}, D_{ow} < 3.08$), demonstrating that the OMCs observed have different affinities to lipid tissues. According to Miller et al.[33] non-ionized compounds with $-1 < \log K_{ow} < 5$ are expected to translocate from roots to other plant tissues, which is consistent with the results obtained for all the neutral compounds identified in this study (Table S5).

Human Exposure and Health-risk Assessment Analysis. Tomato is one of the most important crops around the world, with global production currently around 130 million tons, of which 88 million is destined for the fresh market and 42 million processed. Considering the intensive consumption of tomato worldwide, the evaluation of human OMC exposure when RW is used as irrigation is of particular interest, even more when this assessment focuses on real samples submitted to long-time RW irrigation.

In this study, an assessment of human exposure for each analyte quantified in samples was carried out by the estimation of the daily tomato consumption required to reach TTC levels in adults (average 70 kg) and toddlers (12 kg). All daily intakes were calculated taking into account the worst-case scenario possible. To this aim, a single sampling event with the highest value for TTC estimations was taking into account to provide the most conservative considerations out of this study.

449 Regarding toxicological effects, substances were classified as follows. 'Class I' for
450 chemicals with simple structure and known metabolic pathways leading to innocuous end
451 products showing a low order of oral toxicity. Class II contains substances that are
452 intermediate. Very few compounds are included in this category, which is not very well
453 characterized and even questionable.[34] They have less innocuous structures than those in
454 Class I but they do not contain potentially toxic structural features. Class III contains
455 substances with complex chemical structures that provide no strong initial presumption
456 of safety and indeed may produce a significant toxicity effect, some of them being
457 genotoxic compounds. Examples of Class III are a number of pharmaceuticals and other
458 common used stimulants including, carbamazepine, caffeine, bezafibrate, clofibric acid,
459 ketoprofen, naproxen, and metoprolol.[20] TTC levels of these pharmaceuticals typically
460 reach values of around 1500 ng kg^{-1} b.w. day^{-1}, while the TTC for genotoxic chemicals is
461 only 2.5 ng kg^{-1} b.w. day^{-1} or 0.15 µg $person^{-1}$ day^{-1}.[27,34] Nevertheless, it is important to
462 remark that the consumption of a substance above the estimated TTC level would not
463 imply that there is a toxicological risk. It may even point out a demand for specific toxicity
464 analysis of the compound.
465 Some analytes quantified in tomato samples in this study are classified in Cramer Class
466 III (4-AAA, caffeine, carbamazepine, hydrochlorothiazide and mepivacaine). Regular
467 TTC values for these substances range from 1500 to 1800 ng kg^{-1} b.w. day^{-1}.[35]
468 Venlafaxine and tramadol are categorized as chronic toxic, being their TTC value
469 commonly set in 240 ng kg^{-1} b.w. day^{-1}.[36] Carbamazepine epoxide has potential genotoxic
470 carcinogenicity. Therefore, TTC reported values are between 1.5 and 2.5 ng kg^{-1} b.w. day^{-1}.[37]
471
472 As can be observed in Table 3, the OMC concentrations found require an adult and
473 toddler consumption of tens to hundreds of kg to reach the TTC values in most cases.

Considering a reasonable tomato daily consumption (according to FAP the average is 0.13 kg of tomatoes per adult per day,[38] depending on the dietary habits and country), these results do not bring along a health risk for the consumers.

As carbamazepine epoxide exhibit genotoxic carcinogenicity, it presented the lowest daily consumption of tomatoes per toddler and adult (400 g and 2.5 kg, respectively) to reach the TTC, despite its low concentration in the samples. These results are in agreement with the low amount intake of carrots to reach the estimated TTC reported by Malchi et al.[20]

The results of this presented study clearly indicate that the estimated TTC values do not pose a health risk for any of the substances at the concentrations found. This contributes towards the safe usage of RW for tomato irrigation under the conditions presented even when the worst conditions were taking into account.

Table 3. Health-risk assessment based on TTC levels of the OMCs quantified in tomato samples.

Sampling	S1[a]	S2	S3	S4
Maximum OMC concentration (ng g^{-1}, w.w.) detected in tomato samples				
4-AAA	<LOQ[b]	<LOQ	<LOQ	0.4
Caffeine	<LOQ	1	0.8	<LOQ
Carbamazepine	0.2	0.07	0.1	0.2
Carbamazepine epoxide	0.05	<LOQ	<LOQ	0.07
Hydrochlorothiazide	<LOQ	<LOQ	0.1	0.2
Mepivacaine	<LOQ	<LOQ	<LOQ	0.1
Tramadol	<LOQ	0.2	<LOQ	0.7
Venlafaxine	<LOQ	<LOQ	0.1	0.1
Daily consumption of tomatoes (kg) per adult (70 kg) to reach the TTC values				
4-AAA[c]	-	-	-	315
Caffeine[c]	-	114	150	-
Carbamazepine[c]	548	1800	1260	600
Carbamazepine epoxide[d]	3.5	-	-	2.5
Hydrochlorothiazide[c]	-	-	840	840
Mepivacaine[c]	-	-	-	1400
Tramadol[d]	-	67	-	22
Venlafaxine[d]	-	-	112	140
Daily consumption of tomatoes (kg) per toddler (12 kg) to reach the TTC values				
4-AAA[c]	-	-	-	54

Caffeine[c]	-	20	26	-
Carbamazepine[c]	94	309	216	103
Carbamazepine epoxide[d]	0.6	-	-	0.4
Hydrochlorothiazide[c]	-	-	144	144
Mepivacaine[c]	-	-	-	240
Tramadol[d]	-	67	-	4
Venlafaxine[d]	-	-	19	24

[a]S: sampling event; [b]<LOQ: concentration below the limit of quantification; [c]compound classified according to Munro *et al.* 1996;[35] [d]compound classified according to Houeto *et al.* 2012.[37]

Nevertheless, more studies are needed including the evaluation of exposure to other hazards such as synergistic effects due to the addition of concentrations, mixtures of compounds and the formation of metabolites and transformation products that may be more toxic than the original compound. More information about OMCs identified in real crop samples, agricultural procedures and the consideration of sensitive population groups, should also be evaluated to conclude that reuse of RW in agriculture is a safe approach.

ABBREVIATIONS USED

4-AAA: 4-acetyl-aminoantipyrine

4-FAA: 4-formyl-aminoantipyrine

APCI: Atmospheric pressure chemical ionization

ESI: Electrospray

GH: Greenhouse

IDA: Information dependent acquisition

LOQ: Limit of quantification

OMCs: Organic microcontaminants

PSA: Primary-secondary amine

RSD: Relative standard deviation

RW: Reclaimed water

SP: Soilless perlite culture

TTC: Toxicological threshold concern

ASSOCIATED CONTENT

Supporting Information

Information about experimental details: list of target analytes, LC-MS/MS details; analytical method validation in tomato fruit and leaves information, OMCs found in RW and agricultural soils, physico-chemical properties of compounds detected in samples and extracted ion chromatograms and MS/MS spectra of the identified compounds by suspect screening strategy (PDF).

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REFERENCES

(1) Lavrnić, S.; Zapater-Pereyra, M.; Mancini, M. L. Water Scarcity and Wastewater Reuse Standards in Southern Europe: Focus on Agriculture. *Water. Air. Soil Pollut.* **2017**, 228–251.

(2) Wu, X.; Conkle, J. L.; Ernst, F.; Gan, J. Treated Wastewater Irrigation: Uptake of Pharmaceutical and Personal Care Products by Common Vegetables under Field

Conditions. *Environ. Sci. Technol.* **2014**, *48* (19), 11286–11293.

(3) Christou, A.; Karaolia, P.; Hapeshi, E.; Michael, C.; Fatta-Kassinos, D. Long-Term Wastewater Irrigation of Vegetables in Real Agricultural Systems: Concentration of Pharmaceuticals in Soil, Uptake and Bioaccumulation in Tomato Fruits and Human Health Risk Assessment. *Water Res.* **2017**, *109*, 24–34.

(4) Pan, M.; Wong, C. K. C.; Chu, L. M. Distribution of Antibiotics in Wastewater-Irrigated Soils and Their Accumulation in Vegetable Crops in the Pearl River Delta, Southern China. *J. Agric. Food Chem.* **2014**, *62*, 11062−11069.

(5) Riemenschneider, C.; Al-Raggad, M.; Moeder, M.; Seiwert, B.; Salameh, E.; Reemtsma, T. Pharmaceuticals, Their Metabolites, and Other Polar Pollutants in Field-Grown Vegetables Irrigated with Treated Municipal Wastewater. *J. Agric. Food Chem.* **2016**, *64* (29), 5784–5792.

(6) Vergine, P.; Salerno, C.; Libutti, A.; Beneduce, L.; Gatta, G.; Berardi, G.; Pollice, A. Closing the Water Cycle in the Agro-Industrial Sector by Reusing Treated Wastewater for Irrigation. *J. Clean. Prod.* **2017**, *164*, 587–596.

(7) Jörg E. Drewes, Uwe Hübner, Veronika Zhiteneva, S. K. *Characterization of Unplanned Water Reuse in the EU*; 2017.

(8) *Spanish Royal Decree 1620/2007*; 2007; Vol. 294.

(9) *Proposal 2018/0169/COD*; 2018.

(10) Campos-Mañas, M. C.; Plaza-Bolaños, P.; Sánchez-Pérez, J. A.; Malato, S.; Agüera, A. Fast Determination of Pesticides and Other Contaminants of Emerging Concern in Treated Wastewater Using Direct Injection Coupled to Highly Sensitive Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. A* **2017**, *1507*, 84–94.

(11) Pan, M.; Chu, L. M. Fate of Antibiotics in Soil and Their Uptake by Edible Crops.

562 *Sci. Total Environ.* **2017**, *599–600*, 500–512.
563 (12) Martínez-Piernas, A. B.; Plaza-Bolaños, P.; García-Gómez, E.; Fernández-Ibáñez,
564 P.; Agüera, A. Determination of Organic Microcontaminants in Agricultural Soils
565 Irrigated with Reclaimed Wastewater: Target and Suspect Approaches. *Anal.*
566 *Chim. Acta* **2018**, *1030*, 115–124.
567 (13) Martínez-Piernas, A. B.; Polo-López, M. I.; Fernández-Ibánez, P.; Agüera, A.
568 Validation and Application of a Multiresidue Method Based on Liquid
569 Chromatography-Tandem Mass Spectrometry for Evaluating the Plant Uptake of
570 74 Microcontaminants in Crops Irrigated with Treated Municipal Wastewater. *J.*
571 *Chromatogr. A* **2018**, *1534*, 10–21.
572 (14) Picó, Y.; Alvarez-Ruiz, R.; Alfarhan, A. H.; El-Sheikh, M. A.; Alobaid, S. M.;
573 Barceló, D. Uptake and Accumulation of Emerging Contaminants in Soil and Plant
574 Treated with Wastewater under Real-World Environmental Conditions in the Al
575 Hayer Area (Saudi Arabia). *Sci. Total Environ.* **2019**, *652*, 562–572.
576 (15) Calderón-Preciado, D.; Matamoros, V.; Savé, R.; Muñoz, P.; Biel, C.; Bayona, J.
577 M. Uptake of Microcontaminants by Crops Irrigated with Reclaimed Water and
578 Groundwater under Real Field Greenhouse Conditions. *Environ. Sci. Pollut. Res.*
579 **2013**, *20* (6), 3629–3638.
580 (16) Wu, X.; Ernst, F.; Conkle, J. L.; Gan, J. Comparative Uptake and Translocation of
581 Pharmaceutical and Personal Care Products (PPCPs) by Common Vegetables.
582 *Environ. Int.* **2013**, *60*, 15–22.
583 (17) Carter, L. J.; Williams, M.; Martin, S.; Kamaludeen, S. P. B.; Kookana, R. S.
584 Sorption, Plant Uptake and Metabolism of Benzodiazepines. *Sci. Total Environ.*
585 **2018**, *628–629*, 18–25.
586 (18) Dodgen, L. K.; Ueda, A.; Wu, X.; Parker, D. R.; Gan, J. Effect of Transpiration on

587 Plant Accumulation and Translocation of PPCP/EDCs. *Environ. Pollut.* **2015**, *198*,
588 144–153.
589 (19) Santiago, S.; Roll, D. M.; Ray, C.; Williams, C.; Moravcik, P.; Knopf, A. Effects
590 of Soil Moisture Depletion on Vegetable Crop Uptake of Pharmaceuticals and
591 Personal Care Products (PPCPs). *Environ. Sci. Pollut. Res.* **2016**, *23* (20), 20257–
592 20268.
593 (20) Malchi, T.; Maor, Y.; Tadmor, G.; Shenker, M.; Chefetz, B. Irrigation of Root
594 Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and
595 the Associated Human Health Risks. *Environ. Sci. Technol.* **2014**, *48* (16), 9325–
596 9333.
597 (21) Goldstein, M.; Shenker, M.; Chefetz, B. Insights into the Uptake Processes of
598 Wastewater-Borne Pharmaceuticals by Vegetables. *Environ. Sci. Technol.* **2014**,
599 *48* (10), 5593–5600.
600 (22) Prosser, R. S.; Sibley, P. K. Human Health Risk Assessment of Pharmaceuticals
601 and Personal Care Products in Plant Tissue Due to Biosolids and Manure
602 Amendments, and Wastewater Irrigation. *Environ. Int.* **2015**, *75*, 223–233.
603 (23) Paranychianakis, N. V.; Salgot, M.; Snyder, S. A.; Angelakis, A. N. Water Reuse
604 in EU States: Necessity for Uniform Criteria to Mitigate Human and
605 Environmental Risks. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45* (13), 1409–1468.
606 (24) Alcalde-Sanz, L.; Gawlik, B. M. *Minimum Quality Requirements for Water Reuse*
607 *in Agricultural Irrigation and Aquifer Recharge - Towards a Water Reuse*
608 *Regulatory Instrument at EU Level*; 2017.
609 (25) EFSA; WHO. *Review of the Threshold of Toxicological Concern (TTC) Approach*
610 *and Development of New TTC Decision Tree; Review of the Threshold of*
611 *Toxicological Concern (TTC) Approach and Development of New TTC Decision*

612 *Tree*; 2016.
613 (26) Cramer, G. M.; Ford, R. A.; Hall, R. L. Estimation of Toxic Hazard-A Decision
614 Tree Approach. *Food Cosmet. Toxicol.* **1976**, *16* (3), 255–276.
615 (27) Kroes, R.; Renwick, A. G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma,
616 A.; Schilter, B.; Schlatter, J.; Van Schothorst, F.; Vos, J. G.; et al. Structure-Based
617 Thresholds of Toxicological Concern (TTC): Guidance for Application to
618 Substances Present at Low Levels in the Diet. *Food Chem. Toxicol.* **2004**, *42* (1),
619 65–83.
620 (28) Hu, X.; Zhou, Q.; Luo, Y. Occurrence and Source Analysis of Typical Veterinary
621 Antibiotics in Manure, Soil, Vegetables and Groundwater from Organic Vegetable
622 Bases, Northern China. *Environ. Pollut.* **2010**, *158*, 2992–2998.
623 (29) Riemenschneider, C.; Seiwert, B.; Goldstein, M.; Al-Raggad, M.; Salameh, E.;
624 Chefetz, B.; Reemtsma, T. An LC-MS/MS Method for the Determination of 28
625 Polar Environmental Contaminants and Metabolites in Vegetables Irrigated with
626 Treated Municipal Wastewater. *Anal. Methods* **2017**, *9*, 1273–1281.
627 (30) Riemenschneider, C.; Seiwert, B.; Moeder, M.; Schwarz, D.; Reemtsma, T.
628 Extensive Transformation of the Pharmaceutical Carbamazepine Following
629 Uptake into Intact Tomato Plants. *Environ. Sci. Technol.* **2017**, *51* (11), 6100–
630 6109.
631 (31) Gómez, M. J.; Sirtori, C.; Mezcua, M.; Fernández-Alba, A. R.; Agüera, A.
632 Photodegradation Study of Three Dipyrone Metabolites in Various Water Systems:
633 Identification and Toxicity of Their Photodegradation Products. *Water Res.* **2008**,
634 *42*, 2698–2706.
635 (32) Inoue, J.; Chamberlain, K.; Bromilow, R. H. Physicochemical Factors Affecting
636 the Uptake by Roots and Translocation to Shoots of Amine Bases in Barley. *Pestic.*

637 *Sci.* **1998**, *54*, 8–21.
638 (33) Miller, E. L.; Nason, S. L.; Karthikeyan, K. G.; Pedersen, J. A. Root Uptake of
639 Pharmaceuticals and Personal Care Product Ingredients. *Environ. Sci. Technol.*
640 **2016**, *50*, 525−541.
641 (34) Dewhurst, I.; Renwick, A. G. Evaluation of the Threshold of Toxicological
642 Concern (TTC) - Challenges and Approaches. *Regul. Toxicol. Pharmacol.* **2013**,
643 *65*, 168–177.
644 (35) Munro, I. C.; Ford, R. A.; Kennepohl, E.; Sprenger, J. G. Thresholds of
645 Toxicological Concern Based on Structure-Activity Relationships. *Drug Metab.*
646 *Rev.* **1996**, *28* (1–2), 209–217.
647 (36) Stanard, B.; Dolan, D. G.; Hanneman, W.; Legare, M.; Bercu, J. P. Threshold of
648 Toxicological Concern (TTC) for Developmental and Reproductive Toxicity of
649 Anticancer Compounds. *Regul. Toxicol. Pharmacol.* **2015**, *72* (3), 602–609.
650 (37) Houeto, P.; Carton, A.; Guerbet, M.; Mauclaire, A. C.; Gatignol, C.; Lechat, P.;
651 Masset, D. Assessment of the Health Risks Related to the Presence of Drug
652 Residues in Water for Human Consumption: Application to Carbamazepine.
653 *Regul. Toxicol. Pharmacol.* **2012**, *62* (1), 41–48.
654 (38) Tomato Worldwide Consumption http://www.fao.org/faostat/en/#data/CC.
655

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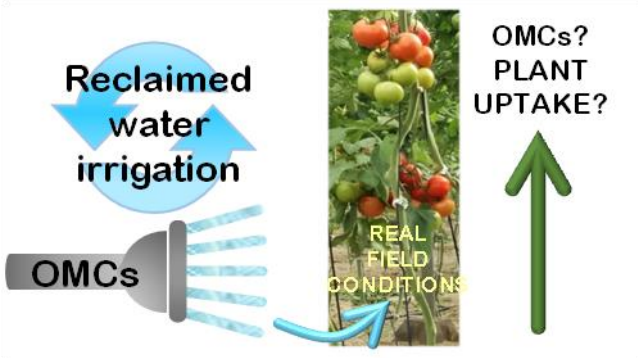

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